Nuclear Pore Complex tethers to the cytoskeleton

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Introduction

There was a time when the nucleus was considered a straightforward protective container for the cell's genes. Later it became evident that the chromosomes had to be organized specifically within the nucleus (Sewitz et al., 2017) and that this organization is dynamic on a long (epigenetic) or short (gene regulation) timescale. An important structure in these processes is the nuclear envelope, a double membrane system, continuous with the endoplasmic reticulum, that encloses the genome and partially separates it from the cytoplasm. The nuclear lamina and other components of the inner nuclear membrane, are primarily involved in epigenetic regulation, whereas the nuclear pore complex (NPC), or proteins of the NPC (nucleoporins) that are not necessarily associated with the NPCs, have roles in short timescale regulation (see Czapiewski et al., 2016 for review).

Recently it has become evident that the role of the nucleus can also extend out into the cytoplasm. To a varying degree, the nucleus is the predominant, and usually by far the most massive, organelle in the cell. It is therefore not surprising that it should have a dominant influence, as well as be used as a platform, for determining cell architecture and function. The LINC complex (LInker of Nucleoskeleton and Cytoskeleton) is the best known anchor of cell architecture to the nucleus (Meinke et al., 2015). It contains SUN domain proteins which are integral membrane proteins, primarily of the inner nuclear membrane, which interact with the nuclear lamina, chromatin and other "nucleoskeletal" structures. The SUN domain of these proteins is in the intramembrane lumen of the nuclear envelope (NE) and binds to KASH domains of the so-called nesprins. Within the LINC complex, nesprins are primarily outer nuclear membrane proteins. There are five human nesprins and multiple isoforms of these (Zhang et al., 2001; Zhang et al., 2005), which differ in their ability to bind to microtubules, F-actin and intermediate filaments. Therefore the primary function of the LINC complex appears to be to link the structural organization of the nuclear interior to the cytoskeleton. Due to the diversity of interactions, as well as the diverse roles of each of the elements of the cytoskeleton (determined by a plethora of binding proteins), the functions of the LINC complex are complex, diverse and poorly understood, but they appear to include mechnotransduction (Davidson and Lammerding 2014; Guilluy and Burridge, 2015), polarity (Neumann and Noegel 2014), and involvement in a variety of diseases (Cartwright and Karakesisoglou 2014)

The other link between the nuclear interior and the cytoplasm is the NPC, the subject of this review. The nuclear membranes present a more-or-less chemically impermeable barrier. Ion channels are present in both inner and outer membranes providing communication between the NE lumen and nucleus or cytoplasm, respectively (Malviya and Klein 2006). NPCs provide a size dependent selective permeability barrier between the nucleus and cytoplasm (Schmidt and Görlich 2016). NPCs allow diffusion (Mohr et al., 2009) of small molecules (solutes to small proteins) but are impermeable to larger macromolecules, unless they bare signals that allow them to bind nuclear transport factors. Transport factors then facilitate movement of primarily RNAs and proteins through the selective barrier by interacting with the FG domains and affecting the functional

organization of the barrier. Directionality in protein, and some RNA, transport is determined by the asymmetrically located Ran system that disassembles import complexes in the nucleus and export complexes in the cytoplasm (Cook et al., 2007). mRNA export is more complex because it is coupled to RNA processing and translation, but similarly involves displacement of transport factors on the cytoplasmic side of the NPC (Delaleau and Borden 2015).

The NPC is arguably the largest protein complex in eukaryotic cells, with an estimated molecular mass of about 100 MDaltons, and 500-1000 individual proteins. It consists of a scaffold, whose structure has been extensively investigated at close to atomic detail (Schwartz 2016). The scaffold consists of a series of stacked rings (Goldberg and Allen, 1996) which stabilize the membrane annulus and provide a platform for organizing the selective barrier. The barrier consists of fully or partially disordered "FG-domains", in the central channel (Lemke 2016). Peripheral structures such as the nucleoplasmic basket and cytoplasmic filaments (Ris, 1989; Goldberg and Allen, 1992; 1993; Fiserova et al., 2014) are also anchored to the nucleoplasmic and cytoplasmic rings, respectively. In lower eukaryotes, such as yeasts, NPCs are thought to be mobile and move in the plane of the double membrane. There movement may be directed by microtubules, at least during cell division (Steinberg et al., 2012). In more complex metazoa, however, interphase NPCs appear to be anchored to the nuclear lamina (Daigle et al., 2001 Fig. 1), a complex intermediate filament network that lines the inner nuclear membrane. How, and whether, NPCs are anchored in plants (Fiserova et al., 2009) and other organisms is not so clear. The nuclear lamina, amongst other roles, is a major component of a mechanically robust inner shell for the NE (Schäpe et al., 2009). Together with other inner nuclear membrane proteins, the lamina also has crucial roles in organizing the interphase chromosomes, such as tethering telomeres, peripheral localization of inactive heterochromatin, determining chromosome and gene positioning and gene activation (Czapiewaki et al., 2016).

Because NPCs appear immobile during interphase (Daigle et al., 2001), and because NPC proteins have been shown to interact with lamins (Smythe et al., 2000), it is assumed that NPCs are fixed in space by stable tethering to the lamina (Moir et al., 2000). Indeed depletion of lamins *in vitro* (Goldberg et al., 1995) and in cells (Guo and Zheng, 2015) leads to NPC clustering and redistribution. However, Nup153, a lamin interacting nucleoporin, is dynamic (Daigle et al., 2001) and is therefore not a good candidate for a protein that maintains a stable interaction. There is also growing evidence for attachments of the NPCs to other cytoskeletal components of the cell, which may be either stable or dynamic. This includes indirect connections via the LINC complex, or direct association of microtubule motor complexes, which mediate the association of NPCs with microtubules. It is these NPC-cytoskeleton linkages that will be the focus of this review.

NPCs and the LINC complex

The relationship between the NPC and the LINC complex has been recently reviewed (Jahed et al., 2016) and therefore will not be the focus of this review

which will instead concentrate on the direct linkages between the NPC and cytoskeleton. However if the LINC Complex does provide an indirect NPC-cytoskeleton association, this must be considered from a functional and experimental point of view. The only published association of LINC complex components with NPC components, is that SUN1 (not SUN2) localizes to the NPCs (Lu et al., 2008) and that SUN1, tagged with GFP at the C-terminus, co-localizes with immunofluorescence of Nup153 (Liu et al., 2007). Nup153 is a nucleoplasmic nucleoporin (Sukegawa and Blobel, 1993; Krull et al., 2004), that may be part of the basket (Fahrenkrog et al., 2002) or nucleoplasmic ring (Walther et al., 2001). Nup153 interacts with, and is recruited to the NPC *in vitro* by lamins (Smythe et al., 2000). Anti-GFP immuno-electron microscopy showed that SUN1-GFP localized very close to the outer edge of the NPC (Liu et al., 2007), consistent with the location of Nup153 (Walther et al., 2001). SUN1 depletion results in clustering of NPCs (Liu et al., 2007), similar to Nup153 depletion (Walther et al., 2001), suggesting that SUN1 is required either for tethering NPCs or to correctly organize NPCs within the NE.

As SUN1 is a component of the LINC complex, and SUN1 binds strongly to KASH domain proteins (Ostlund et al., 2009), it is possible that NPC-associated SUN1 forms a LINC complex that locates one or more elements of the cytoskeleton to the NPC via one or more nesprin isoforms. Although it is reasonable to suppose that at least one nesprin isoform would therefore be located as part of a LINC complex to the NPC, there is no published data to support this and it is possible that SUN1 acts alone, or with different partners, at the NPC.

Surprisingly, Nup153 was also show to interact directly with adenomatous polyposis coli (APC), promoting the association with microtubules in neurons (Collin et al., 2008). This association appears to be at the NE, but presumably Nup153 is, in this case, not located at its normal position on the nucleoplasmic ring/basket, as there is no evidence that microtubules are present in interphase nuclei of metazoa, let alone attached to the nucleoplasmic face of the NPC.

Actin and cytoplasmic intermediate filaments associate with NPCs

Thin section electron microscopy (Gray and Westrum, 1976) and cryo electron tomography (Mahamid et al., 2016) have shown that the cytoskeleton is in close association with the NPCs. Deep etch electron microscopy has also indicated an association of intermediate filaments, possibly vimentin, with the cytoplasmic ring of the NPC (Fujitani et al., 1988). An antibody against a myosin heavy chain-like polypeptide was shown to label NPCs in Drosophila culture cells (Berrios et al., 1991), which could indicate a possible mechanism for linking the NPC to F-actin. However, there is currently little structural, biochemical or functional evidence for such a linkage, other than for nucleoplasmic actin inside amphibian oocyte nuclei (Hofmann et al., 2001; Kiseleva et al., 2004), and for interesting, but unusual, roles in viral transport (Au et al., 2016). Likewise there is some evidence from electron microscopy that cytoplasmic intermediate filaments link to the NPC (Djabali, 1999), but no biochemical or functional data proving such a link. It is possible that close associations between the cytoskeleton and NPC are observed, which do not represent actual binding, because of the crowded nature of the

cytoplasm and the juxtaposition with LINC complexes, known to interact with all elements of the cytoskeleton.

Microtubules are physically linked to the NPC

Electron microscopy clearly shows that microtubules closely associate with NPCs (Gray and Westrum, 1976; Mahamid et al., 2016; Fig. 2) and there is increasing evidence that this is an actual physical linkage via motor proteins, with physiological, variable, complex and not fully understood functions.

Nup358 is a multi-functional platform at the cytoplasmic periphery of the NPC

The microtubule-NPC linkage is best understood in metazoa which possess a cytoplasmically orientated nucleoporin called Nup358 (also known as Ran binding protein 2, RanBP2). Nup358 is a major constituent of the rod-shaped cytoplasmic filaments (Wu et al., 1995; Walther et al., 2002) that emanate from the cytoplasmic ring of the NPC (Goldberg and Allen, 1996) (Fig. 3). It is a large (358 KD), flexible, 36 nm long (Delphin et al., 1997) protein, shown by cross-linking mass spectrometry to be anchored by its N-terminus (Hamada et al., 2011) to two components (Nup133 and Nup96) of the so-called Y-complex (Kosinski et al., 2016), which constitute the scaffolding rings of the core of the NPC. The stable localization of Nup358 to the NPC may also be dependent of Nup214 and Nup88, shown by RNAi experiments in HeLa cells (Bernad et al., 2004), although immuno-depletion of Nup214 from an *in vitro* nuclear reassembly assay showed that Nup214 was not essential for Nup358 localization (Walther et al., 2002).

Nup358 contains several distinct domains (Wu et al., 1995), some of which have related functions and others are seemingly unrelated. It is possible that this large protein, located at the cytoplasmic entrance/exit to the NPC, is a convenient platform to locate disparate processes related to disparate functions of the NPC, such as import, export, RNA processing and control of transcription factors, as well as mediating linkages to microtubules. Most clearly, Nup358 binds to RanGTP as well as to SUMO modified RanGAP. It therefore takes part in the termination of CRM1-mediated export by facilitating the hydrolysis of GTP by Ran, resulting in export complex disassembly (Ritterhoff et al., 2016). Nup358 is itself a SUMO E3 ligase (Pichler et al., 2002), that can, for instance, and for unknown reasons, take part in SUMOylation of Ran (Sakin et al., 2015), but generally, its substrates are poorly understood. Interestingly, SUMO isopeptidases, such as SENP2, which cleave SUMO from the substrate are located at the nucleoplasmic face of the NPC (Zhang et al., 2002), suggesting a role for SUMOylation in the directionality of nucleo-cytoplasmic transport. Therefore, Nup358 is important for the translocation of molecules through the NPC (Singh et al., 1999; Hutten et al., 2008; Wälde et al., 2012; Hamada et al., 2011; Forler et al., 2004; Mahadevan et al., 2013).

The Y-complex and other nucleoporins are important for mitotic microtubule association

One hint that NPC proteins could be linked to microtubules was the discovery that much of the protein transport system as well as major components of the NPC are re-purposed for a seemingly different role in control and assembly of the mitotic spindle (for review see Forbes et al., 2015). Firstly the so called Y-complex (containing Nup107, Nup160, Nup133, Nup96, Nup85, Nup43, Nup37, Sec13 and Seh1) is recruited to kinetochores before attachment of the microtubules can occur (Mishra et al., 2010). Recruitment of the Y-complex enables the subsequent recruitment of the γ-tubulin ring complex (γ-TuRC), which is required for the nucleation of microtubules (Wiese and Zheng, 2006) at centrosomes and kinetochores. Therefore at mitosis the Y-complex can not only be involved in microtubule nucleation but is also necessary for proper spindle formation. Other nucleoporins and transport factors such as ELYS/Mel28, CRM1 and Nup62, controlled by the RanGTP/importin β system are recruited to kinetochores and centrioles and play a role in controlling the assembly of spindle microtubules (Forbes et al., 2015).

Recruitment of Nup358 to interphase NPCs and mitotic kinetochores depends on the Y-complex

One of the first nucleoporins to be discovered at the kinetochore was Nup358 (Joseph et al., 2002). Down-regulation of Nup358 resulted in severely dis-functional kinetochores, including the failure to recruit other essential components (Salina et al., 2003). This suggests that kinetochore-associated Nup358, like the interphase NPC-associated Nup358, is providing a recruitment platform for factors that are essential for functioning of the cellular structure that it is present at, at that time (either the kinetochore or NPC). Kinetochore-associated Nup358 also recruits proteins that it binds to when in the NPC, such as RanGAP1-SUMO1 (Joseph et al., 2004), an association which appears to be negatively controlled by import β (Roscioli et al., 2012)

RNAi down regulation of Nup214 or Nup88 resulted in reduced levels of Nup358 at interphase NPCs (Bernad et al., 2004), although depletion of either in an *in vitro* nuclear assembly assays did not result in loss of Nup358 or the cytoplasmic filaments (Walther et al., 2002). Cross-linking mass spectrometry, combined with cryo-electron tomography, however, indicated that an important anchor for Nup358 could be the Y-complex (specifically Nup133 and Nup96). As with the interphase NPC, the Y-complex is also required for the recruitment of Nup358 to the kinetochore (Zuccolo et al., 2007). Depletion of the Ndc80 complex from the kinetochore resulted in a corresponding reduction of the Y-complex components, Nup133 and Seh1, at the kinetochore. Reduction in Seh1 led to decreased levels of RanGAP1 at the kinetochore, which was presumed to be an effect of mislocalizing Nup358, which is responsible for locating RanGAP1. Conversely, depletion of Nup358 did not affect Nup133 (or presumably the Y-complex).

Not all nucleoporins are recruited to the kinetochore, but a subset, that have links to microtubule assembly and dynamics, do form complexes there, and will now be discussed.

Association of interphase Nup358 with microtubules

Although we have suspected, for at least 40 years, that NPCs associate with the cytoskeleton (Gray and Westrum, 1976), ~~and~~ mechanistic evidence has been lacking. It was realized (Joseph and Dasso, 2008) that although the majority of immuno-fluorescent labeling for Nup358 was at the nuclear envelope, a proportion was clearly present in the cytoplasm. This co-localized with microtubules and could be reduced by RNAi down-regulation of Nup358, proving it was not background labeling. Nup358 down-regulation affected microtubule stability and directional cell migration in CHO-K1 cells. Although such affects were attributed to the cytoplasmic pool of Nup358 which is arranged along the microtubules, it is possible that affects such as disruption of cell polarity, necessary for directed migration, could be attributed to disruption of microtubule-NPC association. In fact disruption of the other known cytoskeleton-NE linkage, the LINC complex, does indeed affect cell polarity and migration (Schneider et al., 2011).

Using immunofluorescence and immuno-precipitation, it was shown that the minus end directed microtubule motor dynein/dynactin complex localized to NPCs and was required for pronuclear migration during mammalian fertilization (Payne et al., 2003). This shows that microtubules do link to NPCs and one, albeit specialized, role is to move nuclei through the cell. However, the exact "cargo" protein was not identified.

The dynein/dynactin complex does not bind directly to cargo molecules, but instead employs adaptor proteins to mediate tethering. One such adaptor is Bicaudal D (BICD). Using a biotinylation proximity assay and mass spectrometry peptide identification, as well as a yeast hybrid assay, Nup358 was identified as a candidate binding partner for BicD2 (Splinter et al., 2010). Co-immunoprecipitation with an anti-Nup358 antibody brought down ~~BICD2~~BicD2, but not other dynein adaptors. Confocal microscopy showed that G2 phase HeLa cells accumulated BicD2 compared to cells at other stages of the cell cycle and that BicD2 immunofluorescence significantly correlated with the localization of NPCs. Knock down of Nup358 appeared to prevent accumulation of BicD2 at the NPCs in G2 cells and knock down of BicD2 resulted in loss of dynein/dynactin from the NPCs.

The same study (Splinter et al., 2010) also indicated that Kinesin1, a plus end directed microtubule motor, may antagonize the action of dynein because it is also located by BicD2 to the NPC. Therefore, at least in G2 phase, a large proportion of NPCs are tethered to microtubules via the dynein/dynactin complex, by the binding of BicD2 to Nup358.

Nuclear lamina prevents microtubule directed movement of NPCs

At this stage, there is not thought to be either any movement of NPCs within the NE, nor of microtubules across the NE surface, nor should there be movement of whole nuclei due to the opposing forces of kinesin and dynein motors. Any movement of NPCs within the plane of the NE is also prevented by their anchorage to the nuclear lamina. Mouse embryonic fibroblasts containing a double lamin

B1/B2 deletion, followed by siRNA knockdown of lamin A/C, resulted in lamin-free cells. This led to an asymmetric distribution of NPCs, which clustered towards the centrosome (Guo and Zheng, 2015), but this only occurred in the G2 phase of the cell cycle, not G1. Disassembly of microtubules or down-regulation of components of the dynein/dynactin motor complex prevented clustering of NPCs. This shows that in these cells the dynein/dynactin minus end directed motor is predominant and that NPCs can be moved towards the centrosome if they are not anchored by the nuclear lamina. The importance of the kinesin counter-force is therefore uncertain, but this clearly shows that NPCs, at least in some cells, and possibly only at particular cell cycle stages are linked to microtubule motors that are functionally attached to cytoskeletal microtubules.

Microtubule attachment to the NPC-associated Y-complex

As mentioned for the kinetochore, Nup358 is not the only microtubule associated nucleoporin: the Y-complex also nucleates microtubules (Mishra et al., 2010). In parallel to this principle, NPC associated Nup133 (part of the Y-complex) also mediates microtubule NPC association, specifically at prophase (Bolhy et al., 2011). Nup133 recruits CENP-F both at the kinetochore (Zuccolo et al., 2007) and the prophase NPC. CENP-F then recruits CENP-E and dynein at the kinetochore (Varis et al., 2006; Mao et al., 2010). Similarly, at the prophase NPC, CENP-F recruits the dynein/dynactin complex via NudE and NudEL. The purpose of these different sequential associations between NPCs and microtubule motor proteins is uncertain, but were suggested to be involved in the correct spatio-temporal positioning of the centrosomes to ensure accurate spindle formation and chromosome separation.

Another interesting example of nuclear movement is in the radial glial progenitor cells involved in neuronal and glial development (Hu et al., 2013). In these cells, nuclei undergo cell cycle dependent movement, and move towards the ventricular surface during G2, and then go through mitosis when the nuclei reach the surface. Interestingly, this movement is centrosome-independent (Tsai et al., 2010), and does not appear to involve the LINC complex (Hu et al., 2013). 3D structured illumination microscopy clearly showed that BicD2 (the Nup358-binding dynein/dynactin adaptor), dynein and dynactin localize to the cytoplasmic face of the NPC in G2, whereas CENP-F (required for Nup133-dependent dynein/dynactin recruitment) only binds later, in prophase. The functional significance of this sequential recruitment is very clear in these cells because RNAi down regulation of BicD2 causes nuclear movement to arrest >30μm from the ventricular surface, whereas depletion of CENP-F allowed them to travel to ~10μm, but not actually reach the ventricular surface. Therefore nuclear migration in G2 depends on the Nup358 linkage, whereas the final steps also include, or switch to Nup133.

Cdk1 controls dynein/dynactin recruitment to NPCs

This seems to be orchestrated by the G2/M phase cyclin dependent kinase, Cdk1 (Baffet et al., 2015). Inhibition of Cdk1 with drugs prevented nuclear migration and inhibited accumulation of dynein at the NE in G2. Conversely, activation of

Cdk1 resulted in premature binding of dynein to the NPCs. The BicD2 binding domain of Nup358 contains Cdk1 consensus sites, and was shown to bind more strongly to BicD2 when phosphorylated by Cdk1/cyclin B. Therefore phosphorylation of this domain by Cdk1 in G2 facilitates BicD2, dynein/dynactin and microtubule attachment to the NPC.

By a different mechanism, Nup133 dynein/dynactin recruitment is controlled by the localization of CENP-F, although this too is controlled by Cdk1. CENP-F is sequestered in the nucleus in G2, but relocates, in a Cdk1-dependent way, to the NPCs in prophase (Baffet et a., 2015). Drug inhibition of G2-phase nuclear migration can be overcome by expression of BicD2 constitutively located to the NE by fusion to the nesprin-3 KASH domain. It is possible, however, that the LINC complex is not able to normally provide this function. Nesprin-1 has been shown to recruit PCM-1 which recruits dynein/dynactin and kinesin complexes to the NE and facilitates nuclear positioning in differentiating myotubes (Espigat-Georger et al., 2016). Nesprin-2 has also been shown to be involved in dynein/dynaction dependent nuclear migration in the retina (Yu et al., 2011). Therefore the LINC complex does mediate nuclear migration. However, the NPC proteins might provide a more flexible platform whose interactions can be orchestrated very precisely as the cell progresses through the stages of G2, prophase and M-phase. Therefore the function of using multiple sequential mechanisms as the cell progresses towards cell division is to provide precise checkpoints in order to ensure the co-ordination and correct timing of multiple processes that must occur for the immensely complex re-organization of the cell during mitosis. Importantly, the functions can also be transferred from the NPC to the kinetochore because unlike LINC complex proteins, these nucleoporins are not integral membrane proteins, and can therefore be assembled into structures not associated with membranes.

Nup358 binds to kinesins

It was discovered that in retina and brain, the kinesin heavy chain microtubule motors, KIF5B and KIF5C, bind to Nup358 at a specific domain (between Ran binding domains 2 and 3) (Cai et al., 2001). It was suggested that the kinesin plus end directed motor could pay a role in delivering cargo to, or taking it away from, the NPC (Mavlyutov et al 2002), a function that may be more crucial in cells with long distance transport requirements, such as neurons. However, it was shown that the kinesin-Nup358 interactions are related to functions away from the NPC in determining the correct localisation and function of mitochondria (Cho et al., 2007). Interestingly, the kinesin binding domain, together with the two flanking ran binding domains, activate the kinesin motor activity of KIF5C (Cho et al., 2009), suggesting that any kinesin bound to Nup358, whether it is in the cytoplasm or at the NPC, would be an active motor. Interaction of Nup358 with kinesin-2 together with APC (adenomatous polyposis coli) was also shown to have a microtubule-dependent role in polarized cell migration (Murawala et al., 2009), although it was suggested that this is a cytoplasmic, rather than NPC-associated, role for Nup358. However, it does indicate a potential mechanism for NPC-microtubule associations.

Conclusion

Nucleoporins have been clearly shown to associate, primarily via motor proteins, to microtubules. The functional significance of this is clearest at the mitotic kinetochore. However roles in nuclear migration, where NPCs appear to act as flexible anchors, have also been demonstrated. It is currently uncertain whether NPC-microtubule linkages are common in different cell types, or whether they only form and function in particular cells, and/or at specific cell cycle stages. Although, for instance, the association between BicD2 and Nup358 is cell cycle regulated, by Cdk1 phosphorylation, it cannot be ruled out that this linkage could be controlled in a similar, or different way, during other cell cycle stages in different cells, perhaps ~~in a~~ less dramatically.

It is interesting to speculate why, in addition to nuclear migration, the cell would attach active microtubule motors to the NPC. One reason could be to move NPCs within the plane of the NE. This can occur if the nuclear lamina is removed: unanchored NPCs migrate when attached to microtubules via BicD2 and the dynein/dynactin complex. The only time when NPCs are naturally unanchored is when they are newly assembled. This is most obvious in telophase/early G1 as the NE reassembles after mitosis. Microtubules could be involved in distributing NPCs evenly, or correctly, as they are assembled.

Another reason could be to link NPCs to the transport highways of the cell (microtubules) in order to efficiently move transport cargoes to and away from the NPC. Much work is required to answer this question, but the possibility is particularly suggested at by the dependence of certain viruses on microtubules for delivery to the NPCs (Campbell and Hope, 2003). Conceptually, diffusion of cargoes to and from the NPC may not be efficient enough in the crowded environment of the cytoplasm. This is particularly true for large cargoes, such as mRNP particles that may be targeted to a specific region of the cell. Interestingly NPCs may also be specifically located to the part of the NE most closely facing the required destination of an mRNA cargo (Colon-Ramos et al., 2003).

Finally, microtubules are rigid rods, and linking this large, usually immobile, organelle (the nucleus) to them, via active motor proteins, could result in a high tensile structure that is rigidly located within the cell. It may be important to maintain the rigidity of the nucleus in order to maintain the organisation of the underlying chromatin, which otherwise could undergo functional alteration in response to physical stresses. Likewise, maintaining the rigid location of the NPCs may be important for the same reason, as there are functional links to active chromatin. As discussed, the NPCs may provide a particularly controllable attachment that could be quickly remodelled in response to signals.

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Figures

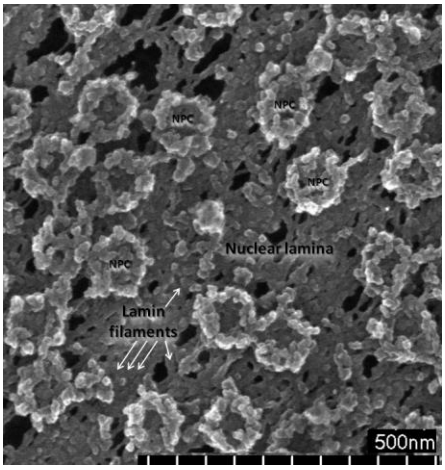

Figure 1. NPCs are anchored to the nuclear lamina. Isolated *Xenopus laevis* nuclear envelope was extracted with non ionic detergent, Triton X-100 to remove the membranes and imaged by high resolution scanning electron microscopy, showing NPCs embedded in and linked to the filamentous lamin network (white arrows).

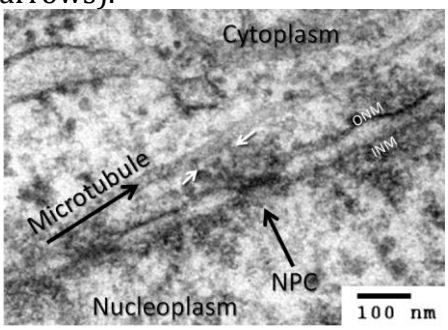

Figure 2. Thin section transmission electron microscopy of MDA MB231 breast cancer cell showing microtubule running parallel to the NE and contacting the cytoplasmic filaments of an NPC (white arrows).

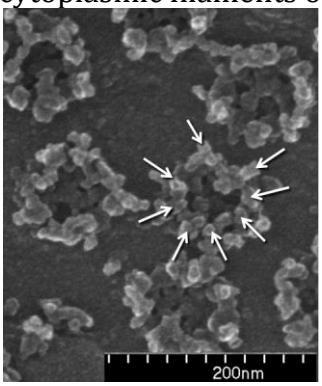

Figure 3. High resolution scanning electron microscopy of isolation Xenopus oocyte NE showing cytoplasmic filaments (arrows) where Nup358 is located.